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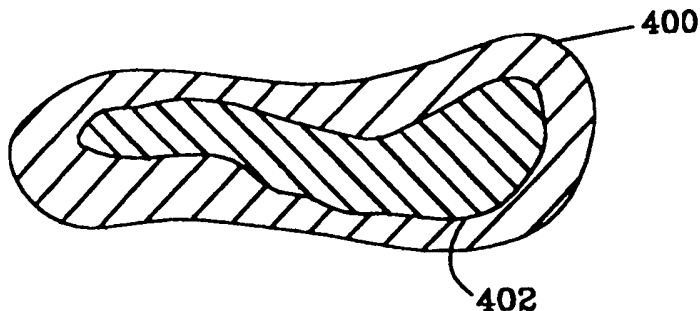
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(54) Title: REVERSIBLE GELING CO-POLYMER AND METHOD OF MAKING

## (57) Abstract

The present invention is a thermally reversible gel or gelling copolymer that is a linear random copolymer of a [meth-]acrylamide derivative and a hydrophilic comonomer, wherein the linear random copolymer is in the form of a plurality of linear chains having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. Addition of a biodegradable backbone and/or a therapeutic agent imparts further utility. The method of the present invention for making a thermally reversible gelling copolymer has the steps of: (a) mixing a [meth-]acrylamide derivative with a hydrophilic comonomer in a solvent with an initiator forming a reaction mixture; (b)

copolymerizing the reaction mixture and forming a first linear random copolymer having a plurality of linear chains having a plurality of molecular weights; and (c) purifying the synthesized first random copolymer and obtaining a second linear random copolymer having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. The gel is enhanced by either combining it with a biodegradable backbone and/or a therapeutic agent in a gelling solution made by mixing the copolymer with an aqueous solvent. Advantages of the present invention include (1) the thermally reversible gel of the present invention exhibits a thermodynamic stability, and when gelled, will not reverse to the liquid state upon dilution but may reverse to the liquid state only in response to a temperature



Moreover, the thermally reversible gel of the present invention has lower

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## REVERSIBLE GELING CO-POLYMER AND METHOD OF MAKING

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### FIELD OF THE INVENTION

The present invention relates generally to a reversible gel and method of making same. More specifically, the gel is a random copolymer of an [meth-]  
10 ]acrylamide derivative with a hydrophilic comonomer. As used herein, the term [meth-]acrylamide denotes methacrylamide, acrylamide, or combinations thereof.

15

### BACKGROUND OF THE INVENTION

Stimuli-sensitive reversible hydrogels are herein defined as copolymer-solvent systems that undergo a transition between a solution and a gel state in response  
20 to the external stimuli such as temperature, pH, ionic strength, solvent composition, sheer stress or a combination of these factors. A reversible stimuli-sensitive gel is one in which the transition is reversed upon reversal of the stimulus. A well known example of a  
25 reversible hydrogel is an aqueous solution of gelatin that is in a solution state at high temperatures (e.g. 80°C) and forms a gel at lower temperatures (e.g., 20°C). Other examples of reversible gels involve aqueous solutions of agarose and kappa-carrageenan that gel in  
30 response to the temperature change, and aqueous solutions of alginate that gel in response to the increased concentration of calcium ions. Reversible hydrogel systems are used in food and pharmaceutical industries as thickeners and suspending agents.

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solutions containing 20 (or more) wt.% of block copolymers of polyethylene oxide and polypropylene oxide, e.g. Poloxamer 407 and Pluronic F68 (Poloxamer 188) exhibit reverse thermal gelation. Solutions of Poloxamer 407 have been investigated for intraocular administration. Solutions containing 25 and 30 wt % of Poloxamer 407 have been prepared and the force needed to inject them through a 25 GA needle was investigated. It was concluded that a liquid-gel transition occurred inside the needle, due to the heat transfer between the needle walls and the surroundings. [J. Juhasz, A. Cabana, A. Ait-Kadi, EVALUATION OF THE INJECTION FORCE OF POLOXAMER 407 GELS FOR INTRAOCULAR ADMINISTRATION, Pharm.Res., 13, No.9, 1996, Symposium Supplement, S-276].

In another example, 25 wt.% aqueous solution of Pluronic F68 was mixed with articular chondrocyte cells suspension at 4°C and injected subcutaneously in nude and immunocompetent rabbit. In both cases, the cells entrapped in the copolymer formed tissue with histological appearance of hyaline cartilage. It was concluded that thermally reversible Pluronic F68 gel can serve as an effective injectable matrix for tissue engineering. [C.A.Vacanti, et al., Proceedings of Tissue Engineering Society, Orlando, FL, 1996]

An example of a pH-reversible hydrogel, investigated as an in situ gelling system for ophthalmic use is the aqueous solution of, a poly(acrylic acid) polymer, which undergoes a pH-mediated phase transition at concentrations above 0.1 wt.%. The solution also contains hydroxypropyl methylcellulose, a viscosity enhancing agent. [Pharm.Res., 13, No.9, 1996, Symposium Supplement].

A new vehicle for topical and mucosal delivery, based on reversible gelation, was developed as an interpenetrating polymer network (IPN) of poly(acrylic

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acid) and a block copolymer of poly(ethylene oxide)/poly(propylene oxide). When heated from ambient to body temperature the network exhibited a significant viscosity increase from a viscous liquid to a gel-like consistency. It was concluded that at higher temperature, reduced release rates of active ingredients from the network were observed due to the increased viscosity of the IPN. [E.S. Ron, et al., A NEW VEHICLE FOR TOPICAL AND MUCOSAL DRUG DELIVERY, Pharm.Res., 13, No.9, 1996, Symposium Supplement, S-299].

All gels containing the copolymers of poly(ethylene oxide)/ poly(propylene oxide), i.e., Poloxamer 407, Pluronic F68 (Poloxamer 188), an IPN of poly(acrylic acid) and a block copolymer of poly(ethylene oxide)/ poly(propylene oxide), and combinations thereof exhibit a limited, concentration dependent, stability of the gel state. The gels formed from these copolymers become liquids upon dilution (as for example due to the dilution with body fluids after peritoneal injection). Additionally, all the above examples of reversible hydrogels exhibit high initial viscosity in a liquid state, i.e., before the gelling transition.

Accordingly there is a need for a reversible gel that only reverses when a specific stimulus is reversed and does not reverse upon introduction of a different stimulus (e.g. dilution). Moreover, there is a need for a reversible gel that has a lower initial viscosity.

The U.S. patent 5,262,055 to Bae et al. discusses an artificial pancreas utilizing reversible gels based on NiPAAM and its copolymers. These polymers and copolymers do not reverse upon dilution and they have a lower initial viscosity. However, the NiPAAM homopolymer

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Accordingly, there remains a need for a thermally reversible gel without substantial syneresis.

Polymers exhibiting phase transitions in water have many potential uses for drug delivery as stated in GRAFT  
5 COPOLYMERS THAT EXHIBIT TEMPERATURE-INDUCED PHASE TRANSITIONS OVER A WIDE RANGE OF pH, G. Chen, AS Hoffman, Nature, Vol 373, 5 Jan 1995 (pp49-52). In this paper, the authors further describe a temperature sensitive polymer that phase separates with a change in temperature  
10 or pH. Chen and Hoffman use graft copolymers having side chains of a temperature sensitive homopolymer, the oligo-N-isopropylacrylamide, grafted onto a pH sensitive backbone homopolymer of acrylic acid. The authors describe the phase separation of the graft copolymer  
15 investigated by a cloud point determination in dilute solutions. However, a dilute solution cannot produce a reversible gelation of these graft copolymers. Chen and Hoffman also mention random copolymers of N-isopropylacrylamide and acrylic acid as exhibiting a  
20 phase separation, however, there is no description of the intention to study the possibility of reversible gelation in more concentrated solutions of these random copolymers.

The reversible gel of the present invention is  
25 useful as a therapeutic agent carrier, for example chemo-embolic material. Chemo-embolic materials are used in treatment of unresectable liver malignancies by a procedure called transcatheter arterial chemo-embolization. The aim of this procedure is to  
30 provide therapeutic embolization of the proper hepatic artery and localize the delivery of chemotherapeutic agents. Currently, the procedure is conducted using iodized oil and small pieces of gelatin foam. These materials are not efficient and research continues for  
35 finding new materials for chemo-embolization.

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Accordingly, there is a need for improved chemo-embolization material(s).

## SUMMARY OF THE INVENTION

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The present invention is a thermally reversible gel or thermally reversible gelling copolymer that is a random copolymer of an [meth-]acrylamide derivative and a hydrophilic comonomer, wherein the random copolymer is in the form of a plurality of linear chains having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. The thermally reversible gelling copolymer is enhanced by either combining it with a therapeutic agent in an aqueous solution containing the thermally reversible gelling copolymer, and/or by grafting the thermally reversible gelling copolymer to a biodegradable backbone.

The method of the present invention for making a thermally reversible gelling copolymer has the steps of:

20 (a) mixing an [meth-]acrylamide derivative with a hydrophilic comonomer in a solvent with an initiator forming a reaction mixture;

(b) polymerizing the reaction mixture and forming a first random copolymer having a plurality of linear chains having a plurality of molecular weights; and

25 (c) purifying the polymerized first random copolymer and obtaining a second random copolymer having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. The method has the further steps of combining the thermally reversible gelling copolymer with either a therapeutic

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Advantages of the present invention include (1) the thermally reversible gel of the present invention exhibits a thermodynamic stability, and when geled, will not reverse to the liquid state upon dilution but may  
5 reverse to the liquid state only in response to a temperature change. Moreover, the thermally reversible gel of the present invention in a solution state has lower initial viscosity more suitable for tissue perfusion.

10 It is an object of the present invention to provide a therapeutic agent carrier.

It is a further object of the present invention to provide a method of making a therapeutic agent carrier.

15 It is a further object of the present invention to provide a biodegradable thermally reversible graft copolymer.

The subject matter of the present invention is particularly pointed out and distinctly claimed in the concluding portion of this specification. However, both  
20 the organization and method of operation, together with further advantages and objects thereof, may best be understood by reference to the following description taken in connection with accompanying drawings wherein like reference characters refer to like elements.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a depiction of a random copolymer of poly(N-isopropylacrylamide-co-acrylic acid) (NiPAAm/AAc),  
30 where n and m denote sequences of NiPAAm and AAc (respectively) that are of random length and are randomly distributed along the copolymer chain.

FIG. 2 is a bar graph of water retention in the gel versus initial copolymer concentration in the gelling  
35 solution.

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FIG. 3 is a graph of fraction of 5-fluorouracil (5FU) released versus time from NiPAAm/AAC copolymer with two different drug loading percentages (20 and 33 wt% of 5FU).

5        FIG. 4a depicts a lymph node sectioned after the injection of thermally reversible copolymer/dye solution.

FIG. 4b depicts another lymph node sectioned after the injection of the dye solution alone.

10                    DESCRIPTION OF THE PREFERRED EMBODIMENT(S)

The present invention is a thermally reversible copolymer that is useful as a gel that forms without substantial syneresis when the thermally reversible  
15 copolymer is in an aqueous solution. Syneresis is defined as water expelled from a copolymer matrix upon gelation. Substantial syneresis is more than about 10 wt% water expelled from the copolymer matrix. According to the present invention, it is preferred that the  
20 syneresis be less than about 10 wt%, more preferably less than about 5 wt% and most preferably less than about 2 wt%. Substantially no syneresis is syneresis of less than about 2 wt%, preferably 0 wt%.

The thermally reversible copolymer is a linear  
25 random copolymer of an [meth-]acrylamide derivative and a hydrophilic comonomer wherein the linear random copolymer is in the form of a plurality of linear chains having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. According to the  
30 present invention, the minimum gelling molecular weight cutoff is at least several thousand and is preferably about 12,000. The presence of a substantial amount of

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amount of hydrophilic comonomer in the linear random copolymer is preferably less than about 10 mole%, more preferably less than about 5 mole% and most preferably about 2 mole%. The structure of linear chains is not cross linked. Moreover, the linear random copolymer structure is one in which a linear chain 100 is shared by randomly alternating portions of the [meth-]acrylamide derivative 102 and the hydrophilic comonomer 104 as depicted in FIG. 1.

10       The [meth-]acrylamide derivative is an N,N'-alkyl substituted [meth-]acrylamide including but not limited to N-isopropyl[meth-]acrylamide, N,N'-diethyl[meth-]acrylamide, N-[meth-]acryloylpyrrolidine, N-ethyl[meth-]acrylamide, and combinations thereof.

15       The hydrophilic comonomer is any hydrophilic comonomer that co-polymerizes with the [meth-]acrylamide derivative. Preferred hydrophilic comonomers are hydrophilic [meth-]acryl- compounds including but not limited to carboxylic acids, [meth-]acrylamide, hydrophilic [meth-]acrylamide derivatives, hydrophilic [meth-]acrylic acid esters. The carboxylic acid may be, for example, acrylic acid, methacrylic acid and combinations thereof. The hydrophilic acrylamide derivatives include but are not limited to N,N-  
20       diethyl[meth-]acrylamide, 2-[N,N-dimethylamino]ethyl[meth-]acrylamide, 2-[N,N-diethylamino]ethyl[meth-]acrylamide, or combinations thereof. The hydrophilic [meth-]acrylic esters include but are not limited to 2-[N,N-diethylamino]ethyl[meth-]  
25       ]acrylate, 2-[N,N-dimethylamino]ethyl[meth-]acrylate, and combinations thereof.

      According to the present invention, the thermally reversible polymer may be mixed with an aqueous solvent to form a thermally reversible gelling solution or  
35       reversible gelling solution. The aqueous solvent includes

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but is not limited to water and aqueous salt solutions. The salt solution is preferably a phosphate buffered saline solution for medical use.

The method of making the thermally reversible  
5 polymer according to the present invention has the steps of:

- (a) mixing an [meth-]acrylamide derivative with a hydrophilic comonomer in a reaction solvent with an initiator forming a reaction mixture;
- 10 (b) polymerizing the reaction mixture and forming a first linear random copolymer having a plurality of linear chains having a plurality of molecular weights; and
- (c) isolating and purifying the polymerized  
15 first linear random copolymer and obtaining a second linear random copolymer having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff.

The alternatives for the [meth-]acrylamide  
20 derivative and the hydrophilic comonomer have been set forth above and are not repeated here.

The reaction solvent may be aqueous or non-aqueous. The preferred aqueous solvent is simply water. Alternatively, the aqueous solvent is a salt solution.  
25 The non-aqueous solvent may be a hydrocarbon including but not limited to oxygenated hydrocarbon solvent, for example dioxane, chlorinated hydrocarbon solvent, for example chloroform, an aromatic hydrocarbon, for example benzene. Precipitation of the polymer occurs during  
30 polymerization in benzene. Dioxane is the preferred solvent because there is no precipitation during copolymerization thereby imparting greater uniformity of

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but may range from about 30 wt% to about 98 wt%. The amount of non-aqueous solvent with respect to the [meth-]acrylamide derivative is preferably about 80 wt% but may range from about 30 wt% to about 98 wt%.

5       The initiator may be any free radical initiator compatible with the [meth-]acrylamide derivative. The preferred initiator is 2,2'-azobis-isobutyronitrile (AIBN). The amount of the initiator with respect to the reaction mixture of solvent and polymer is preferably  
10       about 0.1 wt% but may range from about 0.01 wt% to about 2 wt%.

A reversible gelling solution is made by mixing the thermally reversible polymer with an aqueous solution. The amount of aqueous solution with respect to polymer is  
15       from about 70 wt% to about 99 wt%, preferably about 98 wt% for NiPAAm/AAC to achieve a nonresorbable reversible gel with substantially no syneresis. The aqueous solution is preferably a salt solution.

In addition to the nonresorbable reversible gel  
20       composed of a linear random copolymer of N-isopropyl[meth-]acrylamide and [meth-]acrylic acid described in this invention, a biodegradable (resorbable) copolymer exhibiting similar gelation properties is obtained by grafting of the oligo [meth-]acrylamide  
25       derivative side chains on a biodegradable homopolymer backbone of, e.g., poly(amino acid). Preferred oligo [meth-] acrylamide derivative side chains include N,N-alkyl substituted [meth-] acrylamide derivatives, linear random copolymer of [meth-]acrylamide derivative and  
30       hydrophylic comonomer, and combinations thereof. Techniques of grafting of oligo-N-isopropyl[meth]acrylamide side chains on a nonbiodegradable pH-sensitive homopolymer backbone are described (Chen and Hoffman). The technique(s) of Chen  
35       and Hoffman were used herein to graft the oligo-N-

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isopropyl[meth-]acrylamide side chains on an alternative biodegradable homopolymer backbone such as poly(amino acid). The first step of the synthesis is either the free-radical homopolymerization or the random  
5 copolymerization of the oligo-N-isopropyl[meth-]acrylamide side chains by free radical homopolymerization using an amino-terminated chain transfer agent, for example 2-aminoethanethiol hydrochloride. The next step is the coupling of the  
10 amino-terminated macromer to the carboxyl moieties of the biodegradable backbone using the activation reagent, e.g., dicyclohexyl carbodiimide. Other biodegradable backbones such as poly(phosphazenes) and poly(caprolactone) may also be grafted with the oligo-N-  
15 isopropyl[meth-]acrylamide side chains using similar synthetic techniques. The reaction solvent is non-aqueous, preferably a hydrocarbon, for example chloroform, dichloromethane, N,N'-dimethylformamide or combinations thereof.

20       The resorbable and/or non-resorbable thermally reversible gel(s) of the present invention is/are useful as a therapeutic agent carrier. More specifically, it is useful as a chemo-embolic material by combining the reversible copolymer with a chemo-therapeutic agent  
25 (CTA). At body temperature the reversible copolymer-CTA combination forms a reversible gel matrix containing the entrapped CTA, whereas at room temperature the reversible copolymer-CTA combination is a free-flowing (injectable) solution. The advantages of reversible gels as chemo-  
30 embolizing agents include: fast and effective embolization due to the immediate gel formation at body temperature, and easy incorporation of drugs either by

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therapeutic agent to the reversible copolymer. The localized and controlled release of the CTA entrapped within the gel matrix enhances the efficacy and decreases the systemic toxic effects of chemotherapy.

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Example 1

An experiment was conducted to demonstrate synthesis and thermoreversible gel formation of poly(N-isopropylacrylamide-co-acrylic acid) (NiPAAm/AAC). The  
10 linear high molecular weight NiPAAm/AAC copolymers containing different amounts of AAC were synthesized by a free radical copolymerization.

The [meth-]acrylamide derivative was N-isopropylacrylamide (NiPAAm) (Fisher, Co.) that was  
15 recrystallized from hexane before use. The initiator 2,2'-azobis-isobutyronitrile (AIBN) (Eastman Kodak, Co.) was recrystallized from methanol. The hydrophilic comonomer was acrylic acid (AAC) (Aldrich Co.) that was purified before use by vacuum distillation at 39°C/10  
20 mmHg. The reaction solvent, dioxane, HPLC grade (Aldrich Co.) was used as received. The mixture of [meth-]acrylamide derivative, initiator, hydrophilic comonomer, and solvent formed the reaction mixture.

The molar feed ratio of NiPAAm to AAC was varied as  
25 99:1, 98:2 and 97:3. The copolymerization was carried out in dioxane (80 wt%), with the amount of AIBN initiator of  $1.219 \times 10^{-3}$  mols/L. The reaction proceeded at 60 °C for 18 hours. The resulting copolymer solution was diluted with fresh dioxane and added dropwise to a ten-  
30 fold excess of diethyl ether producing copolymer precipitation. The precipitated copolymer was isolated by filtration and drying. The isolated copolymer was redissolved in acetone and reprecipitated into ten-fold excess diethyl ether. The final, essential step of  
35 purification involved dialysis of aqueous copolymer

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solution through 12,000-14,000 molecular weight cut off (MWCO) dialysis membrane. Dialysis removed the residual unreacted monomer and all copolymer fractions with molecular weights smaller than the MWCO of the dialysis membrane, resulting in a purified copolymer product. The purified copolymer product was further freeze dried.

The removal of molecular weights below 12,000 from the synthesized copolymers was confirmed by gel permeation chromatography. The removal of unreacted monomers was confirmed by nuclear magnetic resonance.

The lower critical solution temperature (LCST) of the synthesized copolymers was evaluated by the cloud point determination method. In this method, 1 wt.% solutions of synthesized copolymers in phosphate buffered saline were heated from 20 to 50°C in 2-deg increments every 10 min. and the absorbance at 450 nm was measured. The cloud point, corresponding to the LCST was determined as the temperature at the inflection point in the absorbance versus temperature curve. NiPAAm homopolymer exhibited an LCST at 32°C. Copolymerization with hydrophilic comonomers shifted the LCST to the physiological temperature range of 36-38 °C. NiPAAm/AAC copolymer containing 2 mol% of AAC exhibited the LCST at 37°C.

Thermally reversible gel formation was studied at 37°C. The freeze dried copolymer was dissolved in phosphate buffered saline (PBS) at different copolymer concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 5.0 wt%) forming copolymer solutions. The PBS was specifically 0.15M NaCl, 0.01 M phosphates  $\text{KH}_2\text{PO}_4$ , and  $\text{Na}_2\text{HPO}_4$ . The copolymer solutions were thermally equilibrated at 37°C for 24 hours. The syneresis (amount of water expelled

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affected by copolymer composition (0, 1, 2 mol% of AAc) and polymer concentration as shown in FIG. 2. In FIG. 2 the amount of water retained in the gel is plotted as a function of the initial copolymer concentration in solution (before gelling). It was unexpectedly discovered that the solution containing at least about 2 wt% of the NiPAAm/AAc copolymer having at least about 2.0 mol % of AAc was able to produce a reversible gel exhibiting substantially no syneresis.

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#### Example 2

An experiment was conducted to confirm the necessity of the minimum gelling molecular weight cutoff. A gelling polymer solution was made as in Example 1, but the solution was not dialyzed so that no low molecular weight species were removed. The result was a solution, milky in appearance, that did not form a gel.

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#### Example 3

An experiment (release study) was conducted to demonstrate that the reversible gel would release a therapeutic agent at a controlled rate.

20

The release study was conducted using NiPAAm/AAc-2 copolymer containing 2 mol% of acrylic acid. Suspensions containing 20 and 33.3 wt% of 5-fluorouracil (5FU) in 5 wt.% copolymer solutions in PBS were prepared at room temperature by mixing and brief sonication. In all suspensions, the 5FU was physically mixed in the suspensions but was not covalently bonded to the copolymer. A 1 ml amount of copolymer/drug suspension was injected into a small dialysis tubing, (d=25 mm and MWCO 12,000-14,000). During the injection, the dialysis tubing was immersed in PBS equilibrated at 37°C. Instantaneous gel formation was observed inside the dialysis tubing. The tubing was then sealed and a gentle

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mixing of the outside solution was turned on. Samples of the outside solution were taken at predetermined time intervals and replaced with the same amount of fresh PBS buffer. Concentration of 5FU was analyzed by UV  
5 spectrometry at 266 nm. The release profiles of 5FU from NiPAAm/AAC-2 copolymer are shown in FIG. 3, where fraction of the released drug is plotted as a function of time.

The release from gels containing 20 and 33 wt.% of  
10 drug were investigated. The release profiles differed markedly in terms of the observed initial burst effect. Within the first 24 hr., the gel containing 20 wt.% of 5FU released almost 40% of drug, whereas the gel containing 33 wt.% of 5FU released less than 15% of drug. Usually,  
15 in the case of drug release from a highly hydrated copolymer matrix the initial release rate is greater for the gels with higher drug loading. To explain this apparent contradiction with the expected results we have to consider the substantial syneresis exhibited by the  
20 gel containing 20 wt.% of drug. In this case, the initial burst effect, normally caused by a fast diffusion from the outer gel layer, was enhanced by the amount of drug expelled from the gel matrix due to the syneresis. After 24 hr., i.e., after the initial burst effect, a  
25 constant release rate was observed for 120 hr for both gels, with a higher release rate observed for the gel containing 20 wt.% loading of 5FU.

#### Example 4

30 A further experiment was conducted to demonstrate the behavior of the gel during tissue perfusion in lymph nodes. A freeze dried copolymer of N-isopropylacrylamide

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copolymer solution. In all solutions, the dye was physically mixed by dissolving into the solutions, but was not covalently bonded to the copolymer.

Canine lymph nodes were freshly isolated and  
5 equilibrated at 37 °C PBS for 30 min.

A 5wt% solution of NiPAAm/AAC in PBS, containing also a small amount ( $>0.01\%$ ) of the blue dye was prepared and cooled in an ice bath. Small aliquots (0.2-0.3 ml) of the cold polymer solution were injected into the  
10 freshly isolated canine lymph nodes. After the injection, lymph nodes were kept at 37°C PBS for 10-15 min permitting the thermal gelation of the injected copolymer solution. The injected lymph nodes were then cut open with a razor blade to evaluate the extent of  
15 tissue perfusion. As shown in FIG. 4a, the dye perfusion within the lymph node 400 was limited to the extent of perfusion of the gelled copolymer solution 402, and was clearly visible.

As a control, dye solution in PBS only was injected  
20 into another lymph node 404 without mixing the dye into the gelling solution. Dye 406 was not contained locally within the lymph node but diffused throughout and beyond the lymph node as illustrated in FIG. 4b. Injection of the dye solution alone resulted in no dye localization  
25 within the lymph node 404.

#### Example 5

An experiment was conducted as in Example 1, but the molar feed ratio of NiPAAm to AAC was varied as  
30 98.4:1.6, 98.2:1.8. These compositions resulted in gelation temperatures of 35°C and 36°C, respectively.

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CLOSURE

While a preferred embodiment of the present invention has been shown and described, it will be  
5 apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. The appended claims are therefore intended to cover all such changes and modifications as fall within the true spirit and scope of  
10 the invention

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## CLAIMS

We claim:

1. A therapeutic agent carrier, comprising:
  - (a) a reversible gelling copolymer, having
  - 5 a linear random copolymer of:
    - (i) an [meth-]acrylamide derivative; and
    - (ii) a hydrophilic comonomer;said linear random copolymer in the form of a plurality of linear chains having a plurality of molecular weights
  - 10 greater than or equal to a minimum gelling molecular weight cutoff;
  - (b) an aqueous solvent mixed with said reversible gelling solution as a reversible gelling solution; and
  - 15 (c) a therapeutic agent mixed with said reversible gelling solution as said therapeutic agent carrier.
2. The therapeutic agent carrier as recited in
- 20 claim 1, wherein an amount of said hydrophilic comonomer in the linear random copolymer is less than about 10 mole%.
3. The therapeutic agent carrier as recited in
- 25 claim 2, wherein said amount is about 2 mole%.
4. The therapeutic agent carrier as recited in claim 2, wherein said amount is from about 1.6 mole% to about 1.8 mole%.
- 30 5. The therapeutic agent carrier as recited in claim 1, wherein said [meth-]acrylamide derivative is selected from the group consisting of N,N'-alkyl substituted [meth-]acrylamides.

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6. The therapeutic agent carrier as recited in claim 5, wherein said N,N'-alkyl substituted [meth-]acrylamide is selected from the group consisting of N-isopropyl[meth-]acrylamide, N,N'-diethyl[meth-]  
5 [meth-]acrylamide, N-[meth-]acryloylpyrrolidine, N-ethyl[meth-]acrylamide, and combinations thereof.

7. The therapeutic agent carrier as recited in claim 1, wherein said hydrophilic comonomer is  
10 hydrophilic [meth-]acryl- compound.

8. The therapeutic agent carrier as recited in claim 7, wherein said hydrophilic [meth-]acryl- compound is selected from the group consisting of carboxylic acid,  
15 [meth-]acrylamide, hydrophilic [meth-]acrylic acid ester, hydrophilic [meth-]acrylamide derivatives and combinations thereof.

9. The therapeutic agent carrier as recited in claim 8, wherein said carboxylic acid is selected from the group consisting of acrylic acid, methacrylic acid and combinations thereof.

10. The therapeutic agent carrier as recited in claim 8, wherein said hydrophilic [meth-]acrylamide derivatives are selected from the group consisting of N,N-diethyl[meth-]acrylamide, 2-[N,N-dimethylamino]ethyl[meth-]acrylamide, 2-[N,N-diethylamino]ethyl[meth-]acrylamide, or combinations  
25 thereof.  
30

11. The therapeutic agent carrier as recited in

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dimethylamino]ethyl[meth-]acrylate, and combinations thereof.

12. The therapeutic agent carrier as recited in  
5 claim 1, wherein said aqueous solvent is selected from the group consisting of water, and aqueous salt solution.

13. The therapeutic agent carrier as recited in  
claim 12, wherein said salt solution is a phosphate  
10 buffered saline.

14. The therapeutic agent carrier as recited in  
claim 13, wherein an amount of said solvent is from about  
70 wt% to about 99 wt%.

15

15. The therapeutic agent carrier as recited in  
claim 1, wherein said therapeutic agent carrier is a  
chemo-embolic material.

20

16. A method of making a therapeutic agent  
carrier, comprising the steps of:

(a) mixing an acrylamide derivative with a  
hydrophilic comonomer in a reaction solvent with an  
initiator forming a reaction mixture;

25

(b) copolymerizing the reaction mixture and  
forming a first linear random copolymer having a  
plurality of linear chains having a plurality of  
molecular weights;

(c) isolating and purifying the copolymerized  
30 first linear random copolymer and obtaining a second  
linear random copolymer having a plurality of molecular  
weights greater than or equal to a minimum gelling  
molecular weight cutoff;

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(d) mixing the thermally reversible copolymer with an aqueous solvent and making a reversible gelling solution; and

(e) adding a therapeutic agent and obtaining  
5 said therapeutic agent carrier.

17. The method as recited in claim 16, wherein said initiator is a free radical initiator.

10 18. The method as recited in claim 16, wherein an amount of said hydrophilic comonomer in the linear random copolymer is less than 10 mole%.

15 19. The method as recited in claim 18, wherein said amount is about 2 mole%.

20 20. The method as recited in claim 16, wherein said [meth-]acrylamide derivative is N,N'-alkyl substituted [meth-]acrylamide.

21. The method as recited in claim 20, wherein said N,N'-alkyl substituted [meth-]acrylamide is selected from the group consisting of N-isopropyl[meth-]acrylamide, N,N'-diethyl[meth-]acrylamide, N-[meth-]  
25 ]acryloylpyrrolidine, N-ethyl[meth-]acrylamide, and combinations thereof.

22. The method as recited in claim 16, wherein said hydrophilic comonomer is hydrophilic [meth-]acryl-  
30 compound.

23. The method as recited in claim 22, wherein

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hydrophilic [meth-]acrylamide derivatives and combinations thereof.

24. The method as recited in claim 23, wherein  
5 said carboxylic acid is selected from the group consisting of acrylic acid, methacrylic acid and combinations thereof.

25. The method as recited in claim 23, wherein  
10 said hydrophilic [meth-]acrylamide derivatives are selected from the group consisting of N,N-diethyl[meth-]acrylamide, 2-[N,N-dimethylamino]ethyl[meth-]acrylamide, 2-[N,N-diethylamino]ethyl[meth-]acrylamide, or combinations thereof.

15  
26. The method as recited in claim 23, wherein said hydrophilic [meth-]acrylic ester is selected from the group consisting of 2-[N,N-diethylamino]ethyl[meth-]acrylate,  
20 2-[N,N-dimethylamino]ethyl[meth-]acrylate, and combinations thereof.

27. The method as recited in claim 16, wherein said reaction solvent is selected from the group  
25 consisting of aqueous solvent, hydrocarbon solvent, and combinations thereof.

28. The method as recited in claim 27, wherein said aqueous solvent is selected from the group  
30 consisting of water, aqueous salt solution and combinations thereof.

29. The method as recited in claim 27, wherein said hydrocarbon solvent is selected from the group  
35 consisting of oxygenated hydrocarbon, chlorinated

- 23 -

hydrocarbon, aromatic hydrocarbon, and combinations thereof.

30. The method as recited in claim 29, wherein  
5 said oxygenated hydrocarbon is dioxane.

31. The method as recited in claim 29, wherein  
said chlorinated hydrocarbon is chloroform.

10 32. The method as recited in claim 29, wherein  
said aromatic hydrocarbon is benzene.

33. The method as recited in claim 16, wherein  
said aqueous solvent is selected from the group  
15 consisting of water, and aqueous salt solution.

34. The method as recited in claim 33, wherein  
said salt solution is a phosphate buffered saline.

20 35. The method as recited in claim 16, wherein  
said therapeutic agent carrier is a chemo-embolic  
material.

36. A biodegradable thermally reversible graft  
25 copolymer, comprising:

(a) a biodegradable polymer backbone; grafted  
with

(b) a side chain selected from the group  
consisting of homo-oligomers of [meth-]acrylamide  
30 derivatives, co-oligomers of [meth-]acrylamide  
derivatives, homo-oligomers of [meth-]acrylamide  
derivatives copolymerized with hydrophilic comonomers,

- 24 -

37. The copolymer as recited in claim 36, wherein said biodegradable copolymer backbone is selected from the group consisting of poly(amino acid), poly(phosphasenes), poly(caprolactone) and combinations thereof.

38. The copolymer as recited in claim 36, wherein said oligo [meth-]acrylamide derivative is an N,N'-alkyl substituted [meth-] acrylamide derivative.

39. The copolymer as recited in claim 36, wherein said oligo [meth-]acrylamide derivative side chain is randomly copolymerized with a hydrophilic comonomer as a linear random oligomer, said linear random oligomer having molecular weight less than a minimum gelling molecular weight cutoff.

40. A reversible gelling copolymer solution, comprising the copolymer as recited in claim 36, mixed with an aqueous solvent.

41. A therapeutic agent carrier, comprising:  
the copolymer solution as recited in claim 40,  
mixed with a therapeutic agent.

42. A method of making a biodegradable thermally reversible copolymer, comprising the steps of:

(a) polymerizing a plurality of side chains selected from the group consisting of homo-oligomers of [meth-]acrylamide derivatives, co-oligomers of [meth-]acrylamide derivatives, homo-oligomers of [meth-]acrylamide derivatives copolymerized with hydrophilic comonomers, co-oligomers of [meth-]acrylamide derivatives copolymerized with hydrophilic comonomers, said side chain having a first active group; and

- 25 -

(b) coupling the side chains to a biodegradable backbone having a plurality of second active groups wherein said first active group connects to one of the plurality of the second active groups.

5

43. The method as recited in claim 42, wherein said biodegradable copolymer backbone is selected from the group consisting of poly(amino acid), poly(phosphazenes), poly(caprolactone) and combinations thereof.

10

44. The method as recited in claim 42, wherein said polymerizing is a free radical copolymerization wherein the first active group is an amino which originates from an amino-terminated chain transfer agent.

15

45. The method as recited in claim 44, wherein said amino-terminated chain transfer agent is 2-aminoethanethiol hydrochloride.

20

46. The method as recited in claim 42, wherein said coupling is with an activation reagent.

47. The method as recited in claim 46, wherein said activation reagent is dicyclohexyl carbodiimide.

25

48. The method as recited in claim 42, wherein said oligo [meth-]acrylamide derivative is an N,N'-alkyl substituted [meth-] acrylamide derivative.

30

49. The method as recited in claim 42, wherein said oligo [meth-]acrylamide derivative side chain is

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having molecular weight less than a minimum gelling  
molecular weight cutoff.

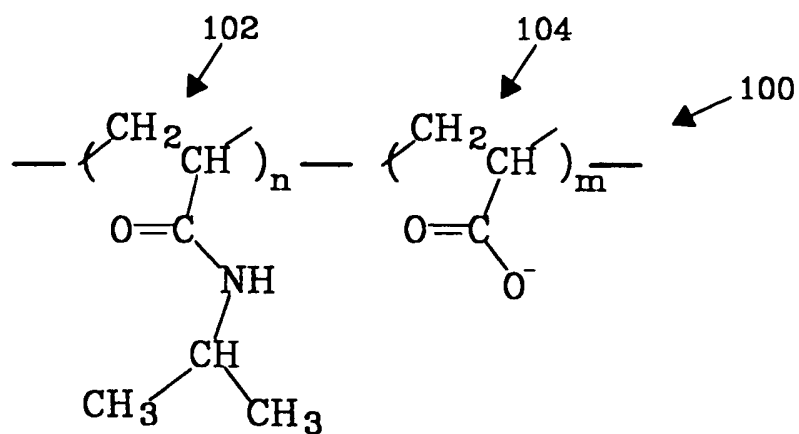
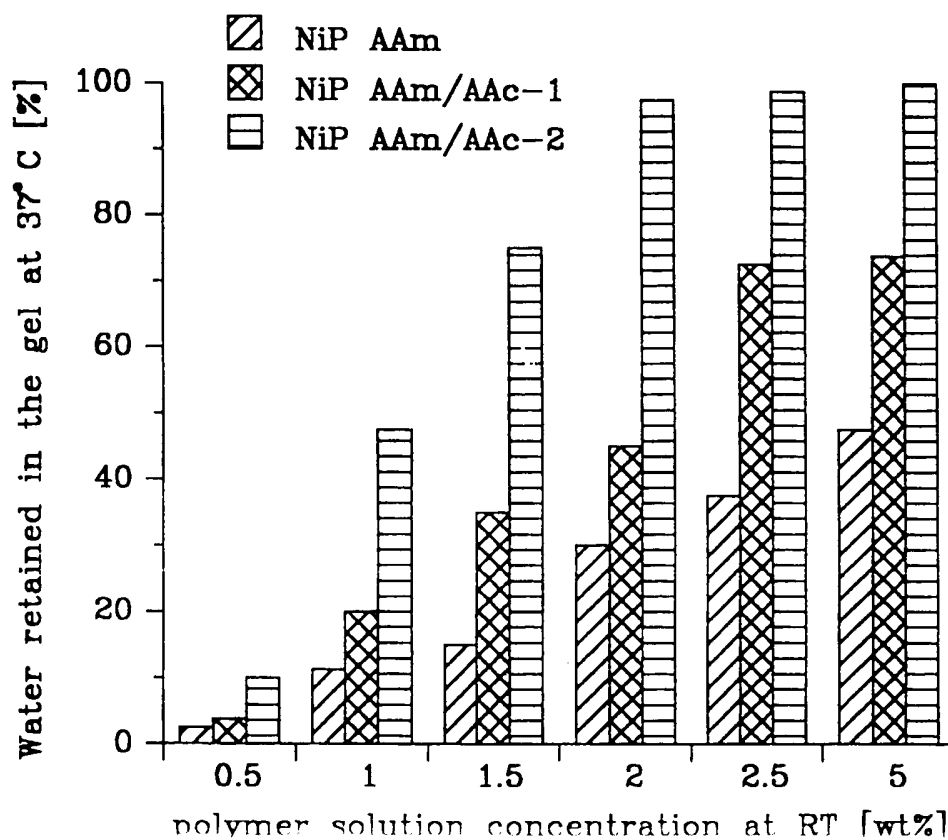
50. The method as recited in claim 42, further  
5 comprising the step of:

mixing the biodegradable copolymer with an  
aqueous solvent.

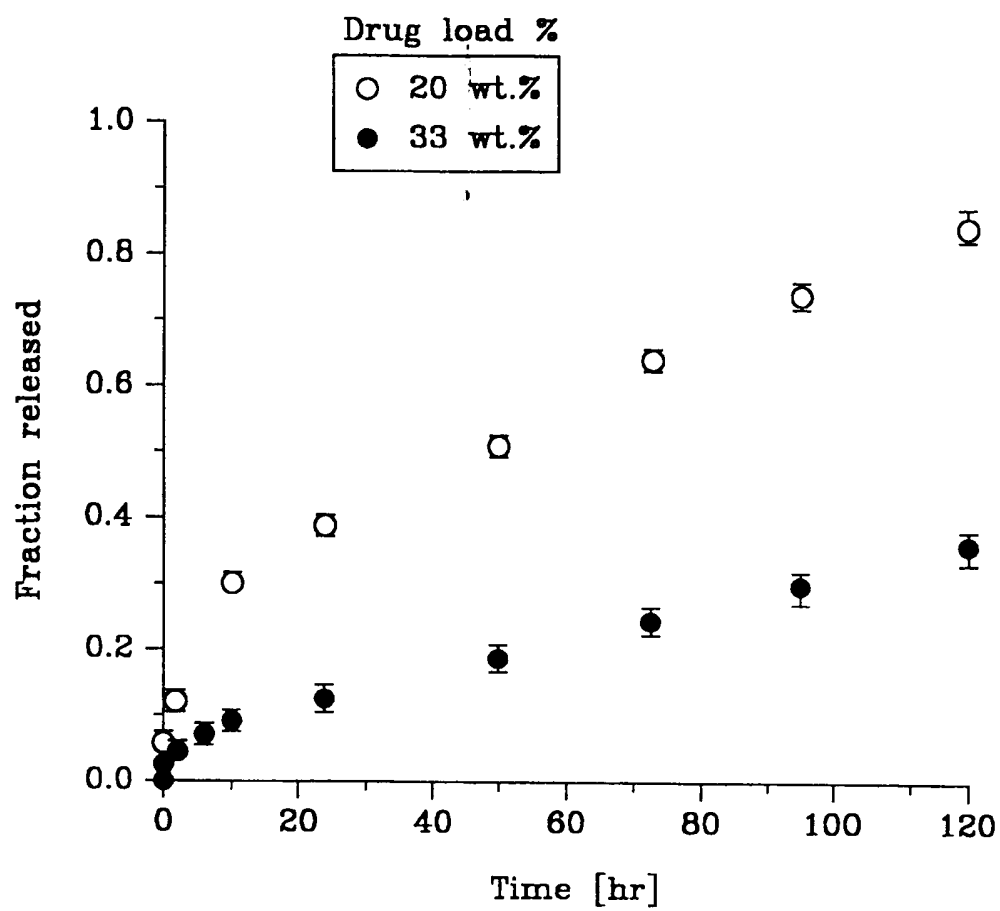
51. The method as recited in claim 50, further  
10 comprising the step of:

adding a therapeutic agent and obtaining a  
therapeutic agent carrier.

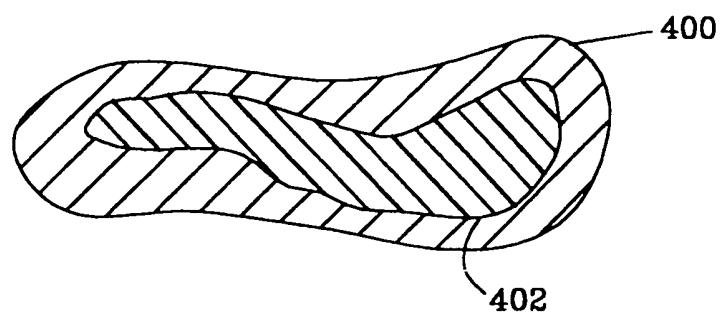
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*Fig. 1*

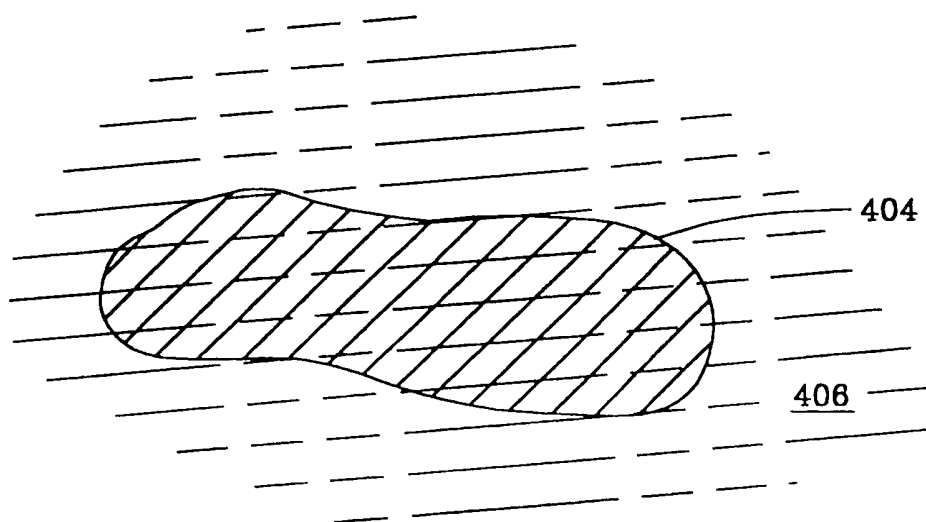
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*Fig. 3*

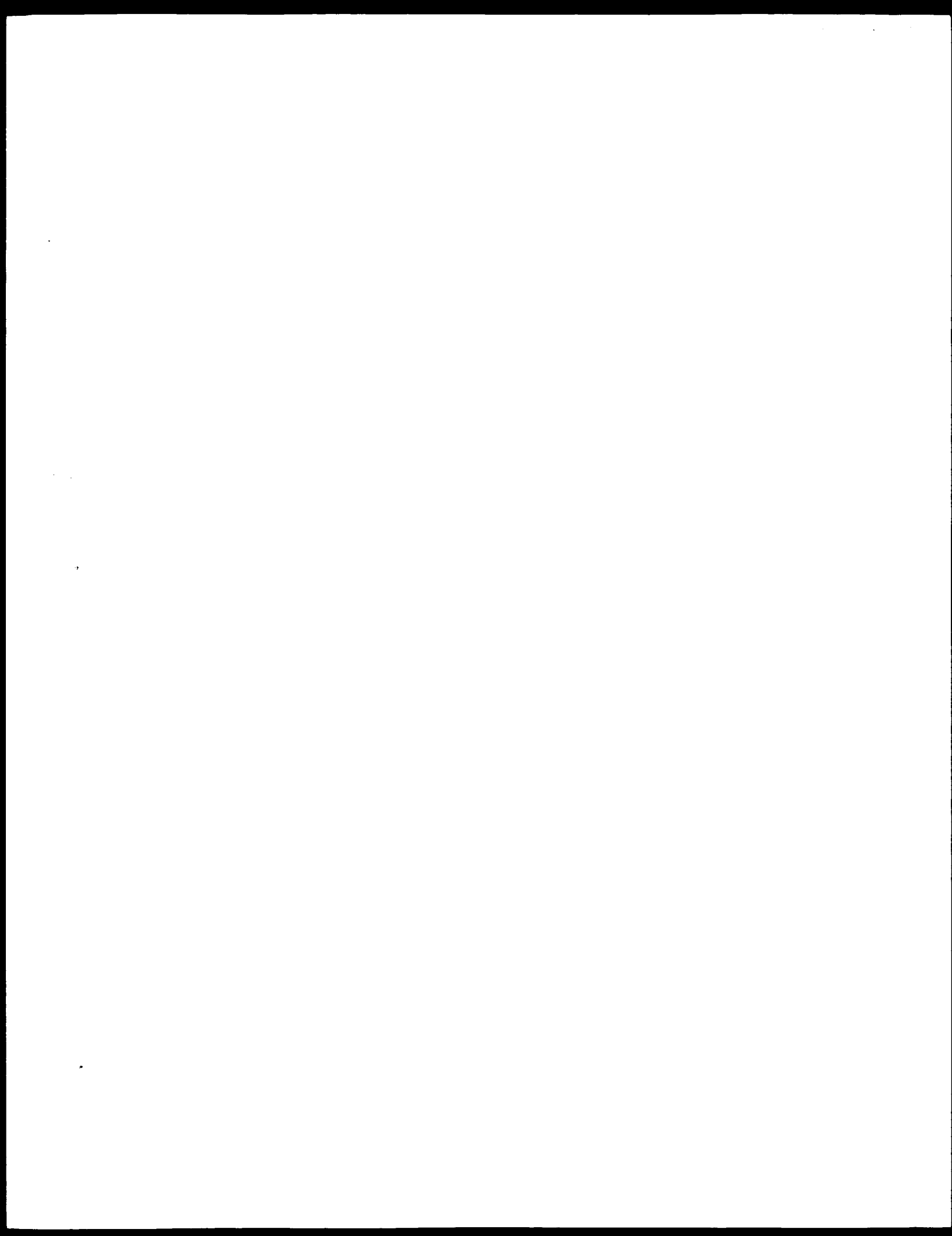
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*Fig. 4a*



*Fig. 4b*





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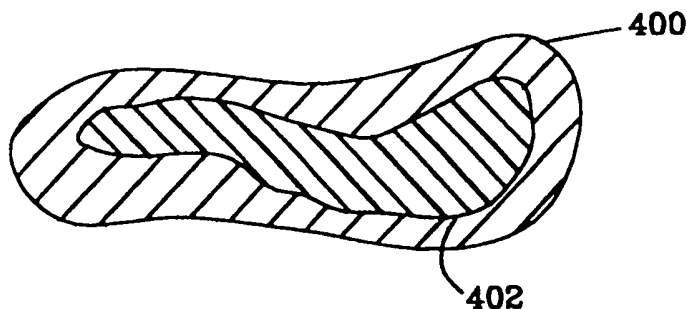
(51) International Patent Classification <sup>6</sup> : <b>A61K 9/20, 47/32, 9/06</b>	<b>A3</b>	(11) International Publication Number: <b>WO 98/55147</b> (43) International Publication Date: 10 December 1998 (10.12.98)
(21) International Application Number: PCT/US98/10964 (22) International Filing Date: 28 May 1998 (28.05.98) (30) Priority Data: 08/870,368                      6 June 1997 (06.06.97)                      US (71) Applicant: BATTELLE MEMORIAL INSTITUTE [US/US]; Pacific Northwest Division, Intellectual Property Services, P.O. Box 999, Richland, WA 99352 (US). (72) Inventor: GUTOWSKA, Anna; 450 Mateo Court, Richland, WA 99352 (US). (74) Agent: ZIMMERMAN, Paul, W.; Battelle Memorial Institute, Pacific Northwest Division, Intellectual Property Services, P.O. Box 999, Richland, WA 99352 (US).	(81) Designated States: CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.          Before the expiration of the time limit for amending the claims          and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 4 March 1999 (04.03.99)	

(54) Title: REVERSIBLE GELING CO-POLYMER AND METHOD OF MAKING

## (57) Abstract

The present invention is a thermally reversible gel or gelling copolymer that is a linear random copolymer of a [meth-]acrylamide derivative and a hydrophilic comonomer, wherein the linear random copolymer is in the form of a plurality of linear chains having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. Addition of a biodegradable backbone and/or a therapeutic agent imparts further utility. The method of the present invention for making a thermally reversible gelling copolymer has the steps of: (a) mixing a [meth-]acrylamide derivative with a hydrophilic comonomer in a solvent with an initiator forming a reaction mixture; (b)

copolymerizing the reaction mixture and forming a first linear random copolymer having a plurality of linear chains having a plurality of molecular weights; and (c) purifying the synthesized first random copolymer and obtaining a second linear random copolymer having a plurality of molecular weights greater than or equal to a minimum gelling molecular weight cutoff. The gel is enhanced by either combining it with a biodegradable backbone and/or a therapeutic agent in a gelling solution made by mixing the copolymer with an aqueous solvent. Advantages of the present invention include (1) the thermally reversible gel of the present invention exhibits a thermodynamic stability, and when gelled, will not reverse to the liquid state upon dilution but may reverse to the liquid state only in response to a temperature change and (2) the gelation occurs without substantial syneresis. Moreover, the thermally reversible gel of the present invention has lower



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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/10964

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K9/20 A61K47/32 A61K9/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	WO 97 00275 A (GEL SCIENCES) 3 January 1997  see claims 1,3,5,7,8,10,25-28,41-44 ---	1,5,7, 16,21, 22,36, 40-42
A	WO 91 15526 A (EASTMAN KODAK) 17 October 1991  see claims 1,9,11,13,14,21,25,26,30 ---	1,5,7, 16,21, 22,36, 40-42
A	WO 91 19481 A (ALLERGAN) 26 December 1991  see claims 1,2,5-7,16,17,25 ---	1,5,7, 16,21, 22,36, 40-42

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	EP 0 534 015 A (GRACE) 31 March 1993  see claims 1-5,11-15 ---	1,5,7, 16,21, 22,36, 40-42
A	US 5 441 732 A (A.L.HOEG ET AL.) 15 August 1995  see claims 1-6,10,18,19,21 ---	1,5,7, 16,21, 22,36, 40-42
A	US 5 262 055 A (Y.H.BAE ET AL.) 16 November 1993 cited in the application ,  see claims 1,9,10 -----	1,5,7, 16,21, 22,36, 40-42

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/10964

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9700275	A	03-01-1997	AU 6385796 A	15-01-1997
			CA 2230727 A	03-01-1997
			EP 0832151 A	01-04-1998
			AU 5523996 A	02-10-1996
			AU 5850196 A	02-10-1996
			CA 2190255 A	19-09-1996
			EP 0759709 A	05-03-1997
			EP 0759710 A	05-03-1997
			WO 9628056 A	19-09-1996
			WO 9628057 A	19-09-1996
WO 9115526	A	17-10-1991	DE 69124722 D	27-03-1997
			DE 69124722 T	04-09-1997
			EP 0476117 A	25-03-1992
			JP 4506378 T	05-11-1992
			US 5432245 A	11-07-1995
WO 9119481	A	26-12-1991	US 5252318 A	12-10-1993
			AT 134508 T	15-03-1996
			AU 8201391 A	07-01-1992
			DE 69117509 D	04-04-1996
			DE 69117509 T	19-09-1996
			EP 0533836 A	31-03-1993
			ES 2083581 T	16-04-1996
			IE 74246 B	16-07-1997
			IL 98320 A	31-10-1995
			PT 97977 A	31-03-1992
			US 5441732 A	15-08-1995
EP 534015	A	31-03-1993	JP 3285903 A	17-12-1991
US 5441732	A	15-08-1995	US 5252318 A	12-10-1993
			AT 134508 T	15-03-1996
			AU 8201391 A	07-01-1992
			DE 69117509 D	04-04-1996
			DE 69117509 T	19-09-1996
			EP 0533836 A	31-03-1993
			ES 2083581 T	16-04-1996
			IE 74246 B	16-07-1997
			IL 98320 A	31-10-1995
			PT 97977 A	31-03-1992
			WO 9119481 A	26-12-1991
US 5262055	A	16-11-1993	AT 162423 T	15-02-1998
			AU 5166193 A	09-05-1994
			CA 2147261 A	28-04-1994
			DE 69316636 D	26-02-1998
			DE 69316636 T	07-05-1998
			EP 0664729 A	02-08-1995
			ES 2111775 T	16-03-1998
			GR 3026399 T	30-06-1998

